

Module 3

Regulation of Gene Expression in Prokaryotes

Recap

- So far, we have looked at prokaryotic gene regulation using 3 operon models.
 - *lac*: a catabolic operon which displays induction via negative control. Also subject to catabolite repression.
 - *trp*: an anabolic operon, which is controlled by repression by negative control, and also has a transcription attenuation system.
 - *ara*: a catabolic operon subject to catabolite repression, and both induction by positive control and negative regulation from the one regulatory protein.
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Prokaryotic Genome Databases

- ❑ Prokaryotic Genome databases & information is available at:
 - GOLD- <http://www.genomesonline.org/>
 - NCBI- http://www.ncbi.nlm.nih.gov/genomes/MICROBES/microbial_taxtree.html
 - DOE-JGI- <http://www.jgi.doe.gov/>
 - TIGR- <http://www.tigr.org/>
 - ❑ PRODORIC database (<http://prodoric.tu-bs.de>)
 - organizes information on prokaryotic gene expression
 - integrates this information into regulatory networks (KEGG) and
 - Online tools to define and predict regulator binding sites
 - ❑ Identification of complete transcription regulatory network
 - <http://bayesweb.wadsworth.org/prokreg.html>
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5.1 Environmental control –Temperature

- The repressors we've looked at modify the availability of the promoter for the RNA polymerase complex – the RNA polymerase holoenzyme is unchanged.
 - RNA polymerase has 5 subunits, with the structure $\alpha_2\beta\beta'\sigma$. The σ subunit (or factor) determines RNA polymerase binding specificity.
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5.1 Environmental control – Temperature.

- ❑ When massive changes in gene expression are required, such as in response to sudden increases temperature (heat shock), a different mechanism is required.
 - ❑ Sudden increases in growth temperature were observed to induce a corresponding increase in the production of certain proteins – the expression of the genes encoding these proteins was induced.
 - ❑ These “heat-shock” proteins are highly conserved in wide ranging members of the domain *Bacteria*.
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5.1 Environmental control – Temperature.

- ❑ So, how is this sudden increase in the expression of heat shock proteins achieved?
 - ❑ In *E. coli*, the promoters of genes encoding heat shock proteins are recognised by a unique sigma factor – σ^{32} .
 - ❑ The σ^{32} /RNA polymerase holoenzyme only recognizes the promoters of heat shock proteins, which differ from σ^{70} -specific promoters in the -35 and -10 boxes.
 - ❑ So heat shock changes the sigma factor in the RNA polymerase, changing the DNA-binding specificity of the holoenzyme.
 - ❑ Additional sigma factors have been identified, controlling functions such as sporulation in *B. subtilis* and phage growth in *E. coli* and *B. subtilis*.
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5.2 Promoter efficiency.

- An alternative mechanism used to regulate RNA transcription is to vary the efficiency with which RNA polymerase binds to the RNA polymerase binding site (the -10 and -35 regions).
 - For example: *lacI*, the gene that encodes *lac* operon regulatory protein, is usually expressed at about 1 mRNA/cell cycle. Promoter sequence mutants can express up to 50 mRNAs/cell cycle, purely on the basis of increased promoter efficiency.
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5.2 Promoter efficiency.

- Promoter efficiency can be controlled either through primary sequence variation, and/or through variation from the 17 bp optimum distance between the -35 and -10 consensus sequences.

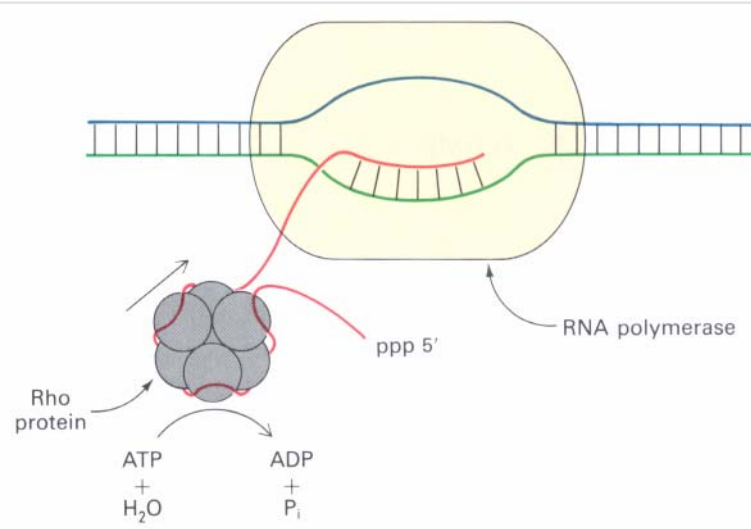
Promoter	-35 region	-10 region
<i>groE</i>	TTTCCCCCTTGAA	CCCCATTTC
<i>dnaK</i> promoter 1	TCTCCCCCTTGAT	CCCCATTTA
<i>dnaK</i> promoter2	TTGGGCAGTTGAA	CCCCTATTA
C62.5 gene	GCTCTCGCTTGAA	CCCCATCTC
σ^{70}	TGCCACCCTTGAAA	GACGATATA
σ^{32} consensus (HS)	T--C-C-CTTGAA	CCCCAT-T
σ^{70} consensus	TTGACA	TATAAT

5.3 Translational control.

- In polycistronic mRNA, the genes located furthest from the promoter are translated less efficiently than those closest to the promoter. This simple phenomenon is known as genetic polarity. There are a number of different mechanisms by which it may occur:
 - The genes at the beginning of an operon are available for translation first, often before distal genes are even transcribed.
 - The translation stop codon of earlier genes can cause the termination of RNA transcription. When translation terminates and ribosomes are released, the ρ transcription termination factor may bind between the stop codon and the RNA polymerase
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5.3 Translational control.

- ρ must bind to the RNA being synthesised to stop transcription. The newly synthesised RNA is normally covered in ribosomes, preventing ρ binding. At translation stop codons, ribosomes are released from the mRNA. This gap between a stop codon and the next ribosome binding site allows opportunities for ρ to bind to the mRNA and terminate transcription. The larger the gap, the more likely this is to occur.



5.3 Translational control.

- ❑ Translation can also be regulated by RNA-RNA hybridisation – antisense RNA.
 - ❑ RNA complementary to the 5' end of mRNA can prevent translation by hybridising to the mRNA. For example, the *E. coli ompF* gene is prevented from being translated by the *micF* antisense RNA. *micF* hybridisation encompasses the SD sequence and the translation initiation codon, effectively blocking ribosome binding.
 - ❑ Antisense RNA is also involved in control of plasmid copy number, and is being investigated for use in gene therapy of cancer and other diseases.
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5.3 Translational control.

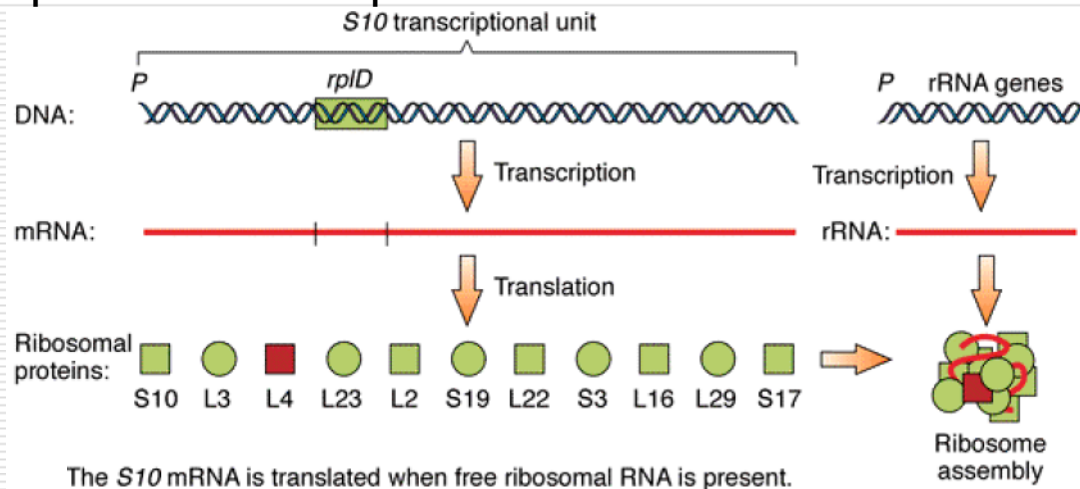
- ❑ Translational initiation efficiency also affects gene expression, in a similar manner to transcriptional efficiency.
 - ❑ Variations in the Shine-Dalgarno sequence lead to different efficiencies of ribosome binding, and this leads to differing translation rates.
 - ❑ Efficient translation initiation site structure:
 - G(GA/AG)G (4-9 nt) AUG
 - Lack of RNA secondary in the region of the RBS or translation start point.
 - AU rich from positions -20 to +13
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5.3 Translational control.

- ❑ Different tRNA's carrying the same amino acid occur at different frequencies within the cell.
 - ❑ Genes with abundant amino acids often have codons that specify common tRNA's.
 - ❑ Genes with low expression may contain several codons that specify rare tRNA's. This leads to a reduced rate of translation as the ribosome pauses while rare tRNA's diffuse to the ribosome.
 - This is similar to the principle by which attenuation structures regulate hairpin-loop formation.
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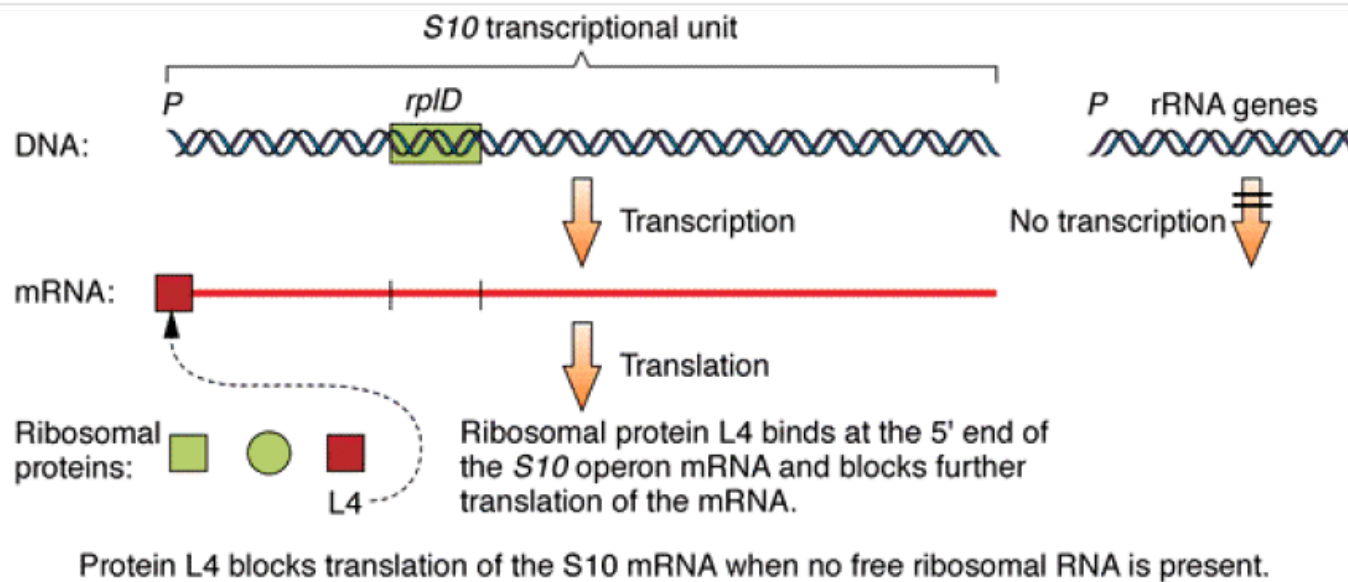
5.3 Translational control.

- ❑ Translation of an mRNA may be inhibited by one of the products it encodes – negative self-regulation or negative autogenous regulation.
- ❑ For example, the *E. coli* S10 transcription unit (mRNA) contains 11 genes for ribosomal proteins. Ribosomal proteins are required when ribosomal RNA (rRNA) is being produced, as both are required to make a functional ribosome.
- ❑ In the presence of rRNA, the product of the third gene (L4) is a component in the production of a new ribosome.



5.3 Translational control.

- In the absence of rRNA, L4 is able to bind to 5' end of the S10 mRNA, preventing further ribosome binding and therefore further translation of the mRNA.



5.4 Post-translational control.

- There is a great deal of variation in the half-life of various proteins. These various half-lives provide another level of control of expression.
 - Two models have been suggested to explain the control of protein degradation in bacteria.
 - The N-end rule
 - PEST hypothesis
 - N-end rule: The amino acid at the N-terminus of a protein act as a signal for proteases that determine the half-life of a protein.
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5.4 Post-translational control.

- For example, modifications of the N-terminal amino acid of the *E. coli* β -galactosidase give the following half-lives.

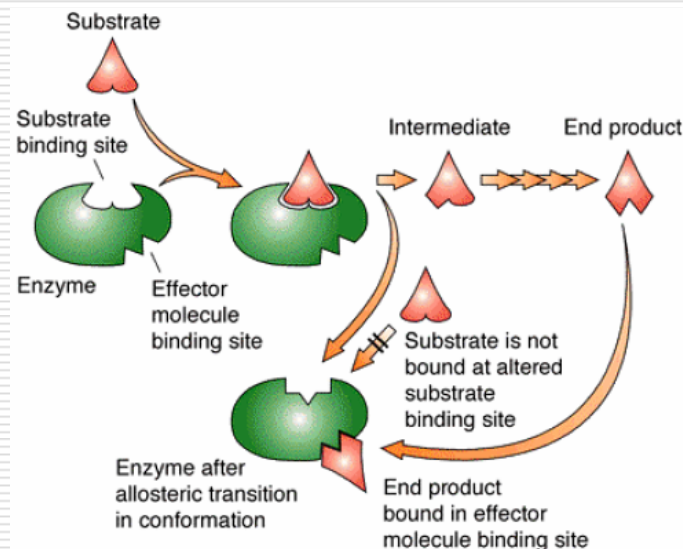
N-terminal amino acid	Half-life
Met, Ser, Ala, Thr, Val, Gly	>20 hours
Ile, Glu	30 min
Tyr, Gln	10 min
Pro	7 min
Phe, Leu, Asp, Lys	3 min
Arg	2 min

5.4 Post-translational control.

- The second model is the PEST hypothesis. Protein degradation is determined by regions rich in one of four amino acids. Proteins with such a region tend to be degraded in less than 2 hours. The four amino acids are:
 - Proline (P)
 - Glutamic acid (E)
 - Serine (S)
 - Threonine (T)
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5.4 Post-translational control.

- Feedback inhibition occurs at the level of enzyme activity. The end product of a biosynthetic pathway inhibits the activity of the first enzyme in the pathway.



5.5 Quorum Sensing: An Activator

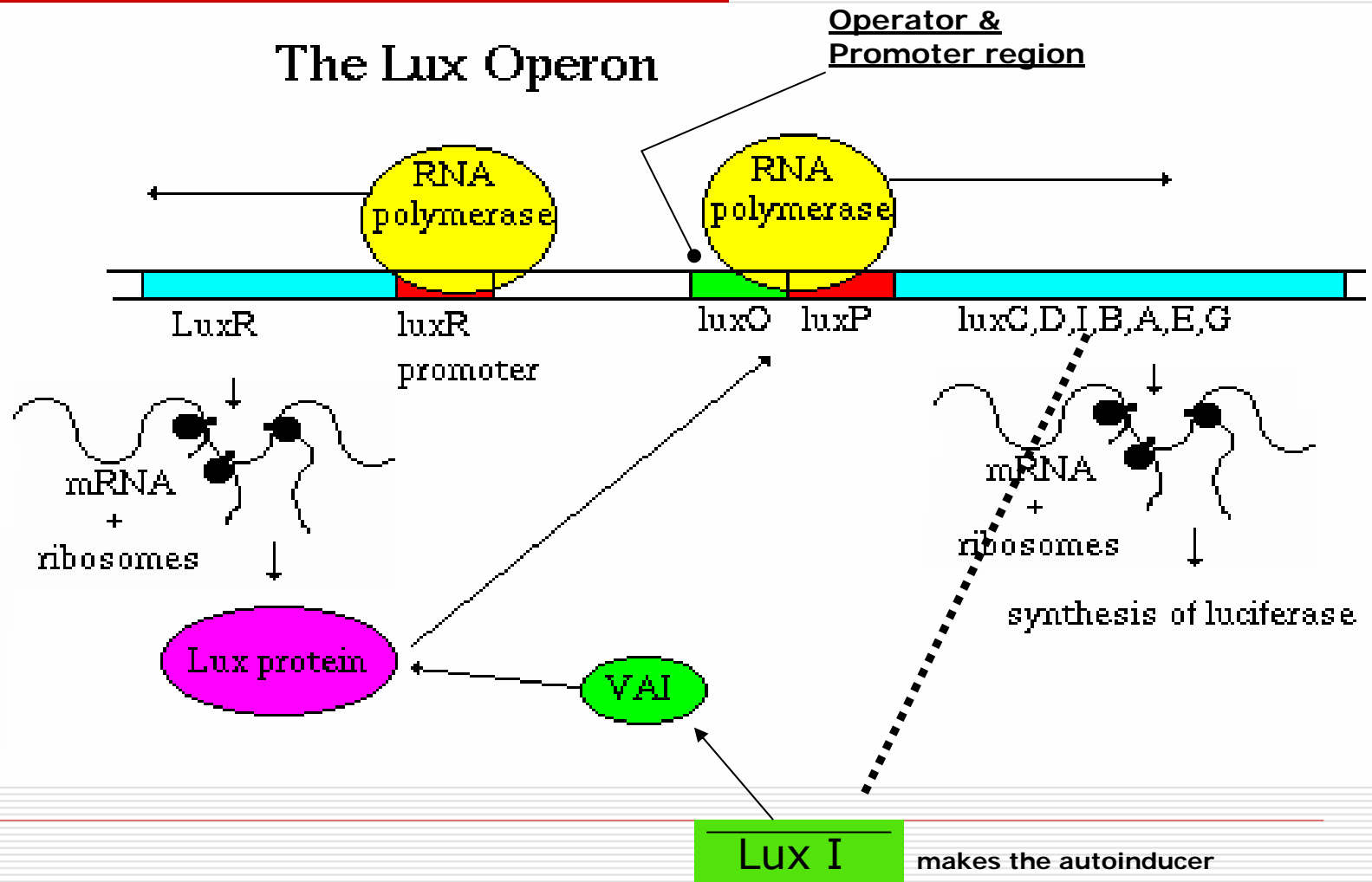
- ❑ A deep ocean squid species swims around at the top of the ocean at night, looking for food.
 - ❑ Problem: To any predator below, this squid appears as a very dark object moving against the very bright background of the moon.
 - ❑ Solving the problem (host): Evolved a **light organ** with pure dense cultures of *Vibrio fischeri* - produces **luciferase** - glows with the same intensity as the moon, rendering the squid invisible to predators from the depths of the ocean. Outside *V. fischeri* is alone, does not make luciferase, does not glow.
 - ❑ Advantage to bacterium: Inside the host, no competition, ready food from the host. Mutualism.
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- Communication between host & bacterium-
Quorum sensing:
 - Very high density is a trigger for the bacterium to assume that they are inside the organ & not alone. Each bacterium continuously secretes a unique small molecule called VAI (*Vibrio fischeri* autoinducer) that can diffuse back into the cell through the cell membrane.
 - Low VAI concentration in more space & less bacteria
 - Increased VAI concentration in confined space & dense culture eg in the organ.
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□ The genes for making luciferase are contained in the lux operon.

- luxO- DNA binding site near the lux promoter (luxP) binds a protein called luxR. This protein somehow calls RNA polymerase over when it is bound to the DNA, thus increasing transcription of the DNA and making more polymerase. Thus, luxR is a **transcriptional activator** of the lux operon.
 - > VAI luxR binds to operator and turns on transcription.
 - < VAI luxR is in a conformation such that it cannot bind to the operator and not very much luciferase can be made.
 - In this fashion, the bacteria only make luciferase when there are lots of other bacteria around. LuxR is consistently transcribed at a low level so that there is always some around to affect regulation.
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The Lux Operon



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- LuxI encodes the enzyme that synthesizes VAI. When a bacterium undergoes the transition from not making luciferase to making luciferase, it needs to have the autoinducer around in order to promote binding of LuxR to the operator. But before the operon is turned on, how can LuxI be made so that there is a continuous level of autoinducer being made? The answer is that operons, in general, are never completely turned off. There is always some **basal level** of transcription going on, but, for example in this case, the uninduced LuxR protein still has a minimal affinity for the DNA binding site so that some DNA can be transcribed to make enough LuxI so that autoinducer is continuously made.
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□ Bacterial cells are single celled organisms but never live individually / independently. independently from each other.

□ Bacteria often communicate with other bacteria in the community. For example, what would be the point of making proteins to carry out bacterial conjugation if there were no other bacteria around?

□ Quorum sensing is used to determine whether or not there are enough bacteria around to make it worth it to turn on the machinery for conjugation, pathogenesis, swarming etc
